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The Effect of Organophosphorus Pesticides on Estuarine Ammonium Oxidizers

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16. ABSTRACT

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The effects of seven thiophosphorus pesticides and their degradation products on estuarine ammonium oxidizers were examined. Using an axenic, closed-culture, high cell density assay, the pesticides caused little inhibition of ammonium oxidation. However, the degradation product of methyl parathion, *p*-aminophenol, significantly inhibited ammonium oxidation in levels as low as 0.01 mg/L. Estuarine sediments containing indigenous ammonium oxidizers were exposed to the pesticides over a 14-day period and activity of the ammonium oxidizers were inhibited after 14 days. Three pesticides were aged in estuarine sediments under aerobic, anaerobic, and microaerophilic conditions, and axenic cultures of ammonium oxidizers were exposed to the resulting metabolites. Ammonium oxidation was inhibited by 10% in the systems containing pesticides aged under aerobic and anaerobic conditions, whereas under microaerophilic conditions, 20% reduction in activity was observed. The data suggest that the parent compounds of the thiophosphorus pesticides have little effect on ammonium oxidation in estuarine sediments, but that certain metabolites which accumulate from the decomposition of these pesticides, especially in sediments under low O_2 levels, may significantly reduce ammonium oxidation.

JONES, R. D., et M. A. HOOD. 1980. The effects of organophosphorus pesticides on estuarine ammonium oxidizers. Can. J. Microbiol. **26**: 1296–1299.

On a évalué l'effet de sept pesticides thiophosphorés et de leurs produits de dégradation sur les agents oxydateurs d'ammonium que l'on retrouve dans les estuaires. A l'aide d'essais en vase clos utilisant une culture axénique à haute densité cellulaire, il est apparu que les pesticides causaient peu d'inhibition de l'oxydation de l'ammonium. Cependant, le produit de dégradation du méthyl parathion, le *p*-aminophénol, inhibe de façon significative l'oxydation de l'ammonium à des concentrations aussi basses que 0,01 mg/L. Des sédiments d'estuaire contenant des agents oxydateurs indigènes ont été mis en contact avec les pesticides pendant une période de 14 jours et l'activité de ces agent oxydateurs s'est trouvée inhibée après 14 jours. Trois pesticides ont été laissés dans des sédiments d'estuaire dans des conditions aérobies, anaérobies et microaérophiles et les cultures axéniques d'agents oxydateurs d'ammonium ont été mises en contact avec les métabolites résultants. L'oxydation de l'ammonium est inhibée de 10% dans les systèmes contenant des pesticides vieillis dans des conditions aérobies et anaérobies, alors que dans des conditions microaérophiles la réduction d'activité est de 20%. Ces résultats laissent croire que les pesticides thiophosphorés, dans leur composition originale, ont peu d'effet sur l'oxydation de l'ammonium dans les sédiments des estuaries mais, par contre, certains métabolites qui s'accumulent par la décomposition de ces pesticides, particulièrement dans les sédiments où la quantité de O_2 est faible pourraient réduire significativement cette oxydation de l'ammonium.

[Traduit par le journal]

Introduction

Information concerning the effects of organophosphorus pesticides on ammonium oxidation appears to be inconsistent (Brown 1955; Garretson and San Clemente 1968; Tu 1970; Gasser 1970; Tu and Miles 1976; Nishio and Kusano 1978). Garretson and San Clemente (1968) observed complete inhibition of nitrification in axenic cultures of *Nitrosomonas europaea* by malathion at 0.01 mg/mL. Likewise, Grant and Williams (G. A. Grant and F. D. Williams. 1979. Abstr. Annu. Meet. Am. Soc. Microbiol. p. 225) observed that parathion at 2.5 mg/L completely inhibited *N. europaea*. However, they also observed no inhibition by the degradation products of the pesticide. In contrast to these findings, Parr (1974) noted that at field rates, organophosphorus insecticides had little effect on ammonium oxidation. Tu (1970) found that several organophosphates at 0.01 and 0.1 mg/g soil caused slight inhibition of nitrification but that activity quickly returned to normal levels due to rapid breakdown of the pesticide.

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With increasing levels of organophosphorus pesticides being used near and subsequently being found in estuaries, the effects of such compounds on nitrification in the estuarine environment is especially relevant. It was, therefore, the purpose of this investigation to examine the effects of seven organophosphates and several of their degradation products on the rate of ammonium oxidation in estuarine sediments. The pesticides chosen were xenobiotes within the "soft," nonpersistant thiophosphorus group which are often used as alternatives to the more persistant, toxic polychlorinated hydrocarbons.

Materials and methods

Axenic culture and pure compounds

The predominant ammonium oxidizers from an estuarine bay, East Bay, Pensacola, FL, were isolated and characterized (manuscript in preparation). The pesticides, methyl parathion, parathion, fonofos, EPN, Guthion®, Abate®, and dicapthon (Chem-Service, West Chester, PA), and the degradation products, methyl paraoxon (MPO), *p*-nitrophenol (PNP), and *p*-aminophenol (PAP) (Fisher Scientific Co., Pittsburgh, PA), were dissolved in acetone (certified, Fisher). The acetone was evaporated in clean sterile tubes for 2 h at 30°C leaving pesticide residues of 0.01, 0.1, 1.0, and 10.0 mg/L as the final concentrations. Controls consisted of acetone alone and no acetone. Ammonium oxidizer medium prepared according to Schmidt et al. (1973) was adjusted to 0.8% salinity with Instant Ocean synthetic sea salts (Aquarium Systems, Inc., Eastlake, OH). A standard inoculum containing 10^7 cells/mL was prepared by growing the isolates in 200 mL of $CaCO_3$-free ammonium oxidizer medium at 30°C for 5 days on a gyrator shaker (model 62, New Brunswick Scientific Co., New Brunswick, NJ) at 100 rpm. The cultures received pH adjustments to pH 8.0 with sterile K_2CO_3 (10%) twice daily. They were filtered through a 0.22-μm filter (Nucleopore Corp., Pleasanta, CA) and resuspended in 100 mL of sterile medium. One millilitre of the standard culture and 9 mL of the medium were added to each tube. After inoculation, the cultures were incubated at 30°C for 24 h and shaken at 30 rpm (model TC-5 tube rotator, New Brunswick). Nitrite concentrations were assayed using the colorometric method of Bendschneider and Robinson (1952). All cultures containing different concentrations of pesticides were prepared in triplicate and each pesticide or degradation compound series was performed in duplicate.

Natural populations and pesticides

Ammonium oxidizer medium (10 mL) was added to methyl parathion, parathion, fonofos, and Guthion® at 10 mg/L and controls prepared as previously described. Sediments, collected from the water–sediment interface using an ethanol-washed corer-type sampler, were stored at 4°C and returned to the laboratory within 2 h. The top 1 cm of the cores was aseptically removed and 1 g was added to each tube. The sediments were incubated at 30°C at 30 rpm and nitrite was determined at 2-, 4-, 6-, 7-, 9-, and 14-day intervals. All cultures were prepared in triplicate and the experiment was repeated twice.

Axenic culture and aged pesticides

Ammonium free basal medium (9 mL) and 1 g of estuarine sediments were added to methyl parathion, parathion, and Guthion® at 10 mg/L and controls prepared as described and allowed to age for 7 days at 30°C under various conditions. Controls were not aged. Aerobic conditions consisted of vigorous rotation at 90 rpm, reduced oxygen conditions consisted of static incubation, and anaerobic conditions consisted of incubation in a Gas Pak (BBL, Cockeysville, MD) whose E_h was maintained below −330 mV. After aging, the media were shaken vigorously for 4 h, ammonium sulfate was added to bring the concentration up to that in the original medium, and the pH was adjusted to pH 7.6. One millilitre of the standard inoculum was added, the cultures were incubated for 24 h at 30°C, and nitrite was determined.

Statistical analysis

Using the standard statistical program, SAS (SAS Institute, Inc., Raleigh, NC), an analysis of variance was performed. A significant effect was established if the calculated F exceeded the probability value at the 95% level of confidence.

Results

The organophosphorus pesticides at concentrations of less than 10.0 mg/L caused no inhibition of ammonium oxidation by the nitrifier in axenic culture. With the exception of the pesticide, fonofos, which caused a significant reduction in activity of 32% and parathion which caused a reduction of 5%, the remaining pesticides at concentrations of 10.0 mg/L had no significant effect on ammonium oxidation (Fig. 1). Similarly, the degradation product of methyl parathion, MPO, had no effect on the nitrifier (Fig. 2), but the compound, PNP, at 10.0 mg/L reduced ammonium oxidation by 25%. The greatest inhibitory effect on the activity of the

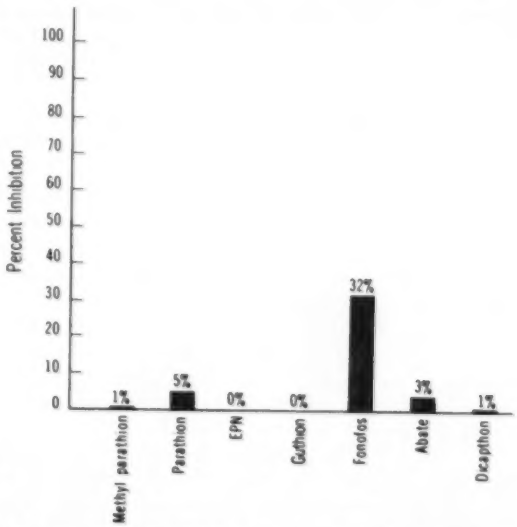

FIG. 1. The effects of seven thiophosphorus pesticides (10.0 mg/L) on ammonium oxidation by the estuarine isolate. Activity is expressed as percent of control levels of nitrite released in 24 h.

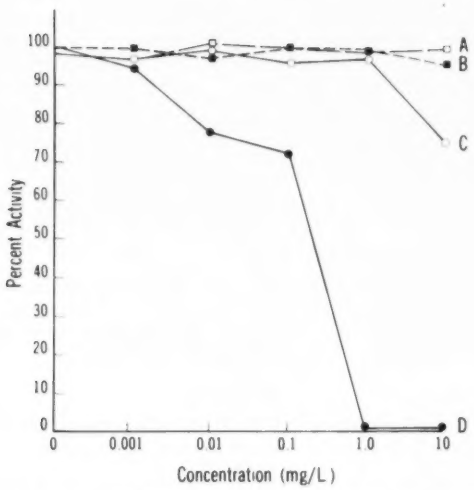

FIG. 2. The effects of methyl parathion and three degradation products on ammonium oxidation by the estuarine isolate. A, parent compound, methyl parathion; B, methyl paraoxon; C, *p*-nitrophenol; D, *p*-aminophenol. Activity is expressed as percent of control levels of nitrite released in 24 h.

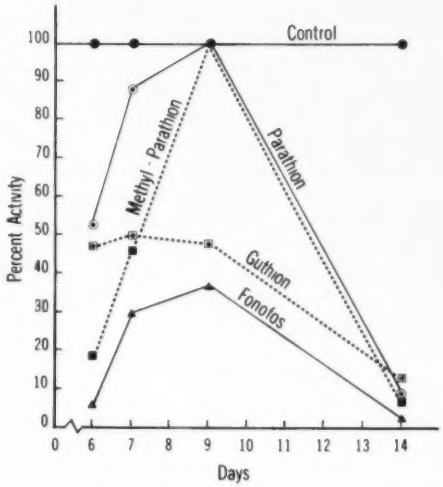

FIG. 3. The effect of four thiophosphorus pesticides on ammonium oxidation by natural estuarine populations of nitrifiers. Activity is expressed as percent of control levels of nitrite released.

organisms was shown by PAP. At concentrations of 0.01 mg/L, ammonium oxidation was reduced by 22%, and at 1.0 and 10.0 mg/L, activity was completely inhibited.

The response of the natural populations of estuarine nitrifiers to 10.0 mg/L of the pesticides (and metabolites which formed) showed variability with time of incubation (Fig. 3). Detectable levels of nitrite were noted in the ammonium-enriched control sediments after 6 days of incubation and continued to rise until day 14. However, the sediments exposed to the pesticide, Guthion®, showed 50% less activity from day 6 to 9, and by 14 days, activity was nearly completely inhibited. Fonofos, methyl parathion, and parathion resulted in a temporal response of activity that was significantly inhibited at day 6 (95, 80, and 50%, respectively), became less inhibited at day 9, and was nearly completely inhibited at day 14. However, unlike fonofos which reduced ammonium oxidation throughout the incubation period, activity in sediments exposed to methyl parathion and parathion returned to control levels at 9 days.

The effects of the aged pesticides on the axenic cultures are shown in Table 1. Methyl parathion, parathion, and Guthion®, aged under aerobic conditions, reduced ammonium oxidation by approximately 10%. Although ammonium oxidation in the anaerobic aged sediments was less than 40% of the activity in the aerobic sediments, which may be due to products of anaerobic metabolism, the effects of the pesticides were relatively similar to those observed under aerobic conditions. Under microaerophilic aging, however, parathion and Guthion® were significantly more toxic, causing 20% reduction in ammonium oxidation.

TABLE 1. Effect of aged pesticide on ammonium oxidation by an estuarine nitrifier[a]

Aging conditions[b]	Nitrite production (mg NO_2 N/L)[c]			
	Acetone control	Methyl parathion	Parathion	Guthion®
Unaged control	86.0	84.1	85.4	84.8
Aerobic	83.7	75.4	75.4	74.4
Microaerophilic	88.4	77.9	69.5	69.2
Anaerobic	35.4	26.9	24.5	27.3

[a]The inoculum was 10^7 cells/mL.
[b]Each pesticide at 10.0 mg/L was aged in 1 g estuarine sediment for 7 days.
[c]Nitrite was measured after 24 h of incubation.

Discussion

Using an axenic culture, high cell density assay, the thiophosphorus pesticides appeared to have little effect on ammonium oxidation with the exception of one compound, fonofos. However, several of the degradation products of methyl parathion and parathion, PNP and especially PAP, inhibited ammonium oxidation at very low concentrations. That certain degradation products of these pesticides reduced ammonium oxidation was further supported by the response of the indigenous sediment populations of ammonium oxidizers exposed to the pesticides over an extended time period. The

pesticides caused inhibition of ammonium oxidation most dramatically at 14 days. This is especially relevant since it has been shown that methyl parathion decomposes in these sediments to PAP–MPO, PNP, and aminomethyl parathion, and after 14 days, concentrations are approximately 22, 16, and 12%, respectively, of the parent compound (H. L. Frederickson. 1979. M.Sc. Thesis, the University of West Florida, Pensacola, FL). From the pure culture data, concentrations approaching 2.2 mg/L PAP and 1.6 mg/L PNP would completely inhibit ammonium oxidation, and, indeed, activity in the sediments was reduced by 95%. Although the decomposition products of Guthion® and fonofos in estuarine sediments are unknown, the data suggest that they are also inhibitory. When such degradation products are identified, further studies are certainly warranted to examine the effect of these metabolites.

It appears that the metabolites which accumulate under microaerophilic conditions have a more toxic effect on ammonium oxidation than those which form aerobically and anaerobically. Munnecke and Hsieh (1976) have shown that under low O_2 levels, parathion is reduced primarily to PAP whereas under aerobic conditions, it is oxidized to PNP. The accumulation of PAP under reduced O_2 conditions may account for the reduction in activity by the ammonium oxidizers exposed to parathion which had been aged under reduced O_2 levels.

The parent compounds of the thiophosphorus pesticides appear to have little effect on the chemolithotrophic process of ammonium oxidation in estuarine sediments. However, the breakdown products of these xenobiotes, especially those formed under reduced O_2 levels, may significantly inhibit ammonium oxidation. Although these compounds are considered short-lived, their metabolites may be much more persistant. For example, PAP has been shown to persist in estuarine sediments in levels as high as 20% of the parent compound for over 50 days (Pritchard et al. 1979). Thus, the metabolites of these pesticides may have a significant impact on the nitrification process in an environment which maintains a very delicately balanced nitrogen cycle.

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